The Central Pressor Effect of Bradykinin in Normotensive and Hypertensive Rats

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SUMMARY The site of action for the pressor response to bradykinin administered into the lateral ventricle has been reported to be either in the septal area or in the ventral portion of the third ventricle. We obtained dose-response curves for the pressor effect of bradykinin injected into the lateral ventricle or the posterior region of the fourth ventricle of normotensive Wistar and spontaneously hypertensive rats (SHR). Responses to fourth ventricle injections had a shorter latency and larger maximal effect, and were 20 to 100 times greater than those to lateral ventricle injections, suggesting that the site of bradykinin's action is in the caudal region of the brain, probably close to the area postrema. Maximal effects were similar for lateral and fourth ventricle injections in both SHR and normotensive rats, but SHR were much more sensitive to bradykinin. The ED50 values for the lateral ventricle route in normotensive rats and SHR were 1.3 and 0.35 nmol, respectively, and, for the fourth ventricle route, 60 and 3.4 pmol, respectively. Responses to Lys-Lys-bradykinin, a kininase-resistant bradykinin analogue, showed that kininase activity is lower in SHR than in normotensive rats and that SHR are four times more sensitive to Lys-Lys-bradykinin than are normotensive rats. The responses of all rats were inhibited by a specific bradykinin receptor blocker [Thi 5-8,DPhe 7]bradykinin. Our results show that there is a site of bradykinin action that is far more caudal than those previously described. The shorter latency and higher sensitivity of the fourth ventricle injection suggest that bradykinin injected into the lateral ventricle diffuses to the fourth ventricle where it exerts its effects. (Hypertension 11 [Suppl I]: I-126-I-129, 1988)

KEY WORDS • bradykinin • central nervous system • pressor response • spontaneously hypertensive rats

THE kinin-kallikrein system is present in the rat central nervous system. The identified components include bradykinin (BK), kallikrein, angiotensin converting enzyme or kininase II, and BK immunoreactive neuronal systems. When injected intracerebroventriculally, BK itself produces a range of effects such as behavioral arousal and sedation accompanied by electroencephalographic alterations, cardiovascular changes, and antidiuretic action. Considerable attention has been given to the pressor effect elicited by BK injected into the lateral ventricle. The site for this hypertensive action has been reported to be the pars ventralis of the lateral septal area. More recently it was demonstrated that access of BK to the third ventricle is essential to the central pressor response to BK, and its site of action has accordingly been ascribed to the periventricular nucleus of the third ventricle. Ventricular plugs occluding the fourth ventricle failed to suppress the central BK pressor response. Earlier reports, however, suggested that the site of the central pressor effect of BK injected in the general circulation of anesthetized rats is the caudal portion of the brainstem. Although these findings have been questioned, recent results obtained in a similar study carried out in dogs suggest that the area postrema is involved in that effect. Since rhomboencephalic structures play an important part in cardiovascular control, it is worth while to investigate whether BK causes a pressor response when injected directly into the fourth ventricle. Injections of BK in the lateral ventricles also caused an increase in the mean arterial pressure of spontaneously hypertensive rats (SHR). The SHR are known to have sympathetic hyperreactivity and, under certain experimental conditions, to present exaggerated responses to BK. We compared the pressor effects of lateral and fourth ventricle injections of BK in normotensive rats (NR) and SHR. We also examined the pressor response to a BK analogue (Lys-Lys-BK), which is resistant to kininase activity, and the effect of a specific BK antagonist [Thi 5-8,DPhe 7]BK. The responses of all rats were inhibited by a specific bradykinin receptor blocker [Thi 5-8,DPhe 7]bradykinin. Our results show that there is a site of bradykinin action that is far more caudal than those previously described. The shorter latency and higher sensitivity of the fourth ventricle injection suggest that bradykinin injected into the lateral ventricle diffuses to the fourth ventricle where it exerts its effects. (Hypertension 11 [Suppl I]: I-126-I-129, 1988)
Materials and Methods

The BK and Lys-Lys-BK used in our study were synthetic products of this laboratory. [Thi<sup>5-8</sup>, tPhe<sup>7</sup>],BK was kindly supplied by Dr. John M. Stewart of the University of Colorado (Denver, CO, USA).

We used 4-month-old, female, normotensive Wistar rats (NR) and 4-month-old, female SHR, all weighing approximately 200 g, bred in this institution. The mean arterial pressures (MAPs) of the NR and SHR were 112 ± 4 and 160 ± 5 mm Hg, respectively. The animals were anesthetized with a mixture of pentobarbital and chloral hydrate. Permanent cannulas were placed in the lateral ventricle (1.6 mm lateral from bregma and 3 mm below skull surface) or in the posterior portion of the fourth ventricle (11.5 mm posterior to bregma and 6 mm deep from skull surface). The cannulas were anchored to the skull by jeweler's screws embedded in dental acrylic cement. After the intracerebroventricular surgery, a polyethylene catheter (PE-10 connected to PE-50) filled with heparinized saline was placed in the abdominal aorta through the left femoral artery. The other end of the cannula was slipped beneath the skin and exteriorized on the back of the animal. After surgery, the animals were individually housed in plastic cages (30 X 20 X 10 cm) that also served as recording chambers.

Two days after implantation the effect of centrally administered BK or Lys-Lys-BK on the MAP was recorded in the unanesthetized and unrestrained animals with a Narco P-100B pressure transducer and a DMP-4B Narco physiograph (Narco Biosystems, Houston, TX, USA). Dose-response curves for BK or Lys-Lys-BK were obtained by injecting 1 µl of saline containing variable concentrations of the peptides at 20-minute intervals and in random order. Three to four doses were administered to each animal.

Proper cannula placement was verified histologically in all subjects by injecting dye at the end of the experiment. The rat brains were excised and placed in 10% formaldehyde for 2 weeks before histology. Lack of diffusion of the dye into the expected ventricular spaces was the criterion for excluding an animal.

Regression lines obtained for the linear parts of the dose-response curves were compared after covariance analyses and tests for regression, linearity, and parallelism. For differences between independent means, Student's t test was used.

Results

Cannula Placement and Injection Diffusion

Dye injected into the lateral ventricle diffused in about 2 minutes into the entire ventricular system, including the contralateral ventricle, the olfactory ventricle, third ventricle, dorsal third ventricle, mamillary recess third ventricle, aqueductus cerebri, the entire fourth ventricle, the medullary central canal, and the ventrolateral and ventral surfaces of the medulla. Dye was also found on the ventral surface of the brain close to the optic chiasm. Animals in which the dye did not spread into the ventricular system did not respond to lateral ventricular BK. One microliter of dye injected into the fourth ventricle stained the posterior region of the fourth ventricle, the ventral surface of the cerebellum, the central canal, and the ventrolateral and ventral surfaces of the medulla.

Effect of Bradykinin in the Lateral Ventricle

Bradykinin, 1 to 10 nmol, injected into the lateral ventricle of NR produced a dose-dependent increase in MAP. The maximum increase in blood pressure of 17 mm Hg was attained with 5 nmol (Figure 1), and the average latency for the manifestation of the pressor response was 90 ± 12 seconds (mean ± SEM). A pressor response was obtained by injecting BK into the lateral ventricle of SHR, in which BK was four times as potent as in NR (see Figure 1). The maximum effect (17 mm Hg) and average latency (94 ± 12 seconds) were the same as obtained for the NR group. The ED<sub>50</sub> values for the BK pressor effect were 0.35 and 1.5 nmol, respectively, for SHR and NR.

Effect of Bradykinin in the Fourth Ventricle

Bradykinin, 10 to 128 pmol, injected into the posterior region of the fourth ventricle of NR also produced a dose-dependent elevation in blood pressure (Figure 2) that typically lasted for 40 to 160 seconds. The overall response pattern was similar to that shown by Lewis and Phillips. The average latency of the pressor response was 2.0 ± 0.3 seconds, which differed (p < 0.001) from the value obtained with BK injected into the lateral ventricle. The ED<sub>50</sub> (60 pmol) was 25 times smaller than that observed for BK injected into the lateral ventricle. The maximum effect on MAP of BK injected into the fourth ventricle (30 mm Hg) was significantly (p < 0.05) larger than that of BK injected into the lateral ventricle.

Bradykinin was 17 times as potent in SHR as in NR. The ED<sub>50</sub> for the response was 3.4 pmol and the maximum pressor effect was at 33 mm Hg (see Figure 2). In SHR, the latency for the BK effect in the fourth ventricle (1.5 ± 0.4 seconds) did not differ significantly (p > 0.05) from that of the NR. The responses to maximally effective doses of BK had durations of 76 ± 12 and 112 ± 20 seconds for SHR and NR, respectively.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Log dose-response curve for the pressor effect of bradykinin (BK) injected into the lateral ventricle of SHR (●) or normotensive rats (NR, ○). The regression lines were homogenous regarding parallelism and differed significantly from each other (p < 0.05, analyses of covariance). Each point represents the mean ± SEM obtained from 8 to 10 rats.
Effect of Lys-Lys-Bradykinin and of a Bradykinin Antagonist

Lys-Lys-BK, 2 to 32 pmol, injected into the fourth ventricle of NR produced a dose-dependent pressor effect similar to that of BK (Figure 3), with an ED$_{50}$ of 6.2 pmol, which represents 10-fold greater activity in relation to that of BK in the fourth ventricle. The maximum effect of Lys-Lys-BK (30 mm Hg) or the average latency (2.2 ± 0.3 seconds) did not differ significantly $(p>0.05)$ from the effect of BK in the same preparation.

The SHR had fourfold greater sensitivity to Lys-Lys-BK in relation to NR. The ED$_{50}$ for Lys-Lys-BK injected into the fourth ventricle of SHR was 1.6 pmol, indicating that the BK analogue was only twice as active as BK injected by the same route. The latency and maximum effect for Lys-Lys-BK in the SHR did not differ $(p>0.05)$ from those in the NR or from those of BK in both NR and SHR.

The effect of the antagonist [Thi$_{5}$-dPhe$_{7}$]BK on BK's pressor action was also analyzed. BK, 125 pmol, injected into the posterior portion of the fourth ventricle of six SHR produced an average pressor response of 35 ± 2 mm Hg (mean ± SEM). When the same dose of BK was injected simultaneously with 1.25 nmol of the antagonist, the average pressor response was 18 ± 2 mm Hg, which differed significantly from control injections $(p<0.001)$.

Discussion

BK injected into the lateral ventricle of NR caused a pressor response with a mean maximal magnitude of 17 mm Hg. Dose-dependent responses were found with 0.5 to 5 nmol of BK, which is within the range of the effective doses used in other reports. The latency for the manifestation of the pressor response to lateral ventricle injection of BK was found to be rather long (average 90 seconds), but is in agreement with the results achieved previously. When injected into the posterior region of the fourth ventricle over the area postrema, BK was 20 times more potent (ED$_{50}$, 60 pmol) and had a shorter latency (2 seconds) and greater maximal effect (30 mm Hg) than when injected into the lateral ventricle. This finding suggests that the site of action of BK is in the caudal portion of the brain, probably near the area postrema. The possibility that BK acts at a more caudal site in the medulla cannot be excluded at present, however. Studies with dye diffusion support the possibility that BK might exert its effect on the ventrolateral or ventral part of the medulla. It is conceivable that the peptide may be carried from the medulla to the ventral portion of the brain by the cerebrospinal fluid. Our results also do not exclude the possibility of other sites (e.g., lateral septal area or periventricular regions of the third ventricle) where BK would exert its effect at higher concentrations and with longer periods of latency.

Our findings apparently contradict the previous evidence that occlusion of the fourth ventricle does not suppress the effect of BK injected into the lateral ventricle. It is possible, however, that BK injected into the lateral ventricle may reach the brainstem by a route other than the one discussed, possibly by leaking from the ventral part of the brain into the subarachnoid fluid. The dose of BK employed was 80 times larger than that necessary to induce a similar effect in the fourth ventricle. Thus if 1.3% of the BK injected into the lateral ventricle reaches the caudal area of the brain, a significant pressor response could occur. The data on the occlusion of the ventral portion of the third ventricle do show, however, that the lateral septal area is not involved in the pressor response to BK injected into the lateral ventricle.

The pattern of the response of the SHR to lateral ventricular BK was similar to that of the NR: the latency and maximal effect were similar; however, BK was...
four times more potent in SHR. The SHR were also much more sensitive (17-fold) to BK injected into the fourth ventricle, although the latency and maximal effect were similar to those observed in the NR.

The greater sensitivity of SHR to BK might be attributed to differences in sensitivity or to a difference in central kininase activity. To elucidate this point we employed a synthetic BK analogue (Lys-Lys-BK) that is resistant to kininase activity. Lys-Lys-BK was 10 times as potent as BK in the fourth ventricle of NR and only twice as potent as BK in SHR. This result suggests that cerebral kininase activity in SHR is greatly reduced in comparison to that in NR. This conclusion is valid if we accept that the central BK receptors in SHR are not different from those of NR. The potency ratio for the Lys-Lys-BK in the NR and SHR groups was 4, indicating that hypertensive rats are more sensitive to the agonist’s action.

Our data show that BK induces a pressor response at a site far more caudal than the one previously described, and the short latency observed after the fourth ventricle injection suggests that the site for the central BK pressor response is in the brainstem, close to the fourth ventricle. The nucleus tractus solitarii does not appear to be involved in the pressor response to BK, since microinjections of the peptide into that nucleus did not cause cardiovascular alterations. The SHR have a markedly reduced kininase activity and, in addition, are more sensitive to BK than are NR. The increased sensitivity to BK could be due to possible changes in receptor sensitivity, sympathetic hyper-reactivity, or differences in vascular responsiveness to sympathetic stimulation in the SHR.

References
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